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EXTRACTION OF LIPIDS FROM BLOOD PLASMA AND SUBSEQUENT INTRODUCTION OF AUTOLOGOUS DELIPIDIZED PLASMA INTO THE BODY AS A POSSIBLE MEANS TO TREAT ATHEROSCLEROSIS

(Experimental Study)

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In recent years different methods of reducing the lipid concentration in the blood have begun to be developed together with diet therapy and the use of hypolipidemic preparations. These include replacement of large volumes of plasma of the patient with donor plasma having a normal cholesterol concentration or plasma protein fractions consisting mostly of albumin and not containing cholesterol [14].

This method is intended for the treatment of patients in whom ischemic heart disease is occurring on a background of hypercholesterolemia that does not lend itself to treatment by diet or hypolipidemic preparations. By repeated replacement of large volumes of autologous plasma with donor plasma or plasma protein fractions in two patients with homozygous familial hypercholesterolemia, a significant reduction of cholesterol concentration in blood plasma and low-density lipoproteins and an increase in the concentration of high-density lipoproteins was achieved, which was accompanied by an improvement in the overall condition and disappearance of stenocardia attacks [14].

Shortcomings of the method include the need to use large amounts of donor plasma, as well as the possibility of the formation of allotypes of lipoproteins during multiple intravenous administrations of homologous donor plasma [4]. The appearance of lipoprotein allotypes can lead to the formation of autoimmune complexes that include the corresponding allotype as an antigen, which according to the antiimmune theory of pathogenesis of atherosclerosis has an unfavorable effect on the course of this disease [1].

A procedure is described in the present study for the extraction of lipids from autologous blood plasma of animals (rabbits, dogs) with subsequent intravenous administration of the delipidized plasma and restoration of erythrocytes.

MATERIAL AND METHODS

The experiments were conducted on male rabbits and dogs.

In one series of experiments (direct development of the atherosclerotic process), we used seven rabbits (three experimental and four control) weighing 1.5 to 2 kg. All the animals received 500 mg cholesterol dissolved in 5 mL sunflower oil daily for 3 months through a stomach tube. Intravenous (through the ear vein) administration of autologous delipidized plasma was carried out twice a week with simultaneous restoration of erythrocytes in the experimental rabbits on a background of an atherogenic diet for 2 weeks after the beginning of the experiment. Such administration was carried out for 3 months, which amounted to a total of 22 administrations in each experimental animal. Intravenous administration of physiological saline was carried out in the control animals.

In a second series of experiments with a group of rabbits weighing 2.5-3 kg with experimental atherosclerosis (the animals received 1 g cholesterol every week for 3 months with vegetables), we chose four rabbits with roughly the same level of cholesterol in the blood serum (1420-1470 mg%). Intravenous administration of delipidized plasma two to three times a week was carried out in two of these rabbits. A total of 15 administrations of delipidized plasma over 2 months were carried out in each animal. Two rabbits served as control (spontaneous regression of the atherosclerotic process) and received an intravenous administration of physiological saline.

The procedure of delipidization of the blood plasma (lipoextraction) in all cases was conducted according to the same scheme. 20 mL of blood were taken from the ear vein of the rabbits. EDTA (1 mg/mL) was used as an anticoagulant. The plasma (12-14 mL) was separated from the erythrocytes by centrifuging at 1500 rpm for 30 minutes. The obtained erythrocytes were immediately mixed with 10 mL physiological saline and returned to the rabbits intravenously. The precooled plasma was then subjected to delipidization by extraction of the lipids with diethyl ether freed of peroxide in a 1:15 ratio [5]. Delipidization was performed on a special apparatus that ensures rotation of the sample at a rate of 12 rpm at 4°C for 16 to 20 hours. After delipidization, the ether phase was separated by centrifugation and discarded, the lower fraction (delipidized plasma) was drawn off with a syringe with a long needle by puncturing the intermediate gel-like layer. After delipidization, the plasma was subjected to dialysis against a large volume of 0.9% sodium chloride solution at 4°C for 10 to 12 hours, then brought to room temperature and administered intravenously to the rabbits.

The concentration of cholesterol and triglycerides in the blood plasma was determined on an AA-2 autoanalyzer from the Technikon Co., the content of phospholipids was determined by the colorimetric method [15]. By determining the difference in absolute content of cholesterol, triglycerides, and phospholipids in the plasma before and after delipidization, we found the amount of lipids separated from the plasma. The percentage of lipid removal in the plasma subjected to delipidization averaged 80 for cholesterol, 94 for triglycerides, and 31 for phospholipids.

The advantage of the method of "mild" delipidization consists of the fact that mostly cholesterol and triglycerides are extracted with it, and only one-third of the phospholipids. This suggests that mostly lipoproteins of very low density enriched with triglycerides and lipoproteins of low density enriched in cholesterol are subject to delipidization, while the high-density lipoproteins rich in phospholipids and having anti-atherogenic properties are extracted to a much lesser degree [6,11].

To investigate the effect of multiple intravenous administration of delipidized plasma, we also used dogs (males weighing 14 kg and females weighing 17 kg). A permanent silicone catheter was introduced into the jugular vein of the animals 1 week before the beginning for the delipidization according to the method of A. M. Korshak and S. D. Groisman [2]. Six procedures of delipidization of plasma at one-week intervals were conducted in each dog. 120 mL of blood (in the morning on an empty stomach) were collected once from the dog and mixed with 30 mL of solution 7b used to take blood from donors. The temporary blood loss was partially compensated by an infusion of physiological saline and glucose. Immediately after centrifuging, the erythrocytes were separated from the plasma, mixed with physiological saline, and returned to the animals, then the plasma (70-90 mL) was subjected to delipidization according to the method just described. 1 mL of a 0.25% solution of levomycetin was added to the delipidized plasma; the plasma infusion was terminated by introducing 10 mL of a 10% urotropin solution to the catheter. The animals received periodic injections of Bicillin 3 (200,000 U each).

The total cholesterol and triglyceride content and also high-density lipoproteins were periodically determined in the blood plasma of the dogs [10]. The total content of cholesterol of the low- and very low-density lipoprotein fractions was found according to the difference between the content of total cholesterol and high-density lipoprotein. Moreover, we followed the dynamics of a number of biochemical indices in the blood plasma of the dogs, reflecting the state of metabolism in the body: content of bilirubin [13], uric acid [8], urea [7], glucose [9] and aspartate-aminotransferase activity [12].

RESULTS AND DISCUSSION

In preliminary experiments, we found that during the perfusion of an isolated, atherosclerotically damaged rabbit aorta [3] with delipidized rabbit plasma for 8 hours, and also during 24-hour incubation of endothelium (turned inside out and tied on the ends) of the atherosclerotic rabbit aorta with delipidized plasma, it was possible to remove a small amount of cholesterol from the aorta (0.44-1.9 mg). These data served as an additional basis for investigating the effect of the repeated intravenous administration of autologous delipidized plasma on the level of blood lipids and the state of the arterial wall in animals with experimental atherosclerosis.

In a series of experiments to investigate the effect of lipoextraction of plasma on the direct development of the atherosclerotic process (Table 1), we demonstrated that repeated administration of delipidized plasma did not reduce the degree of hypercholesterolemia in rabbits. The average level of triglycerides in blood serum in the experimental animals toward the end of the experiment was somewhat lower than in the control animals, but significant individual variations of triglycerides in both groups make this difference insignificant.

Table 1. Effects of lipoextraction of blood plasma on cholesterol and triglyceride concentration in blood serum and degree of atherosclerotic damage of the aorta in rabbits during direct development of the atherosclerotic process.

Number of rabbits	Experimental conditions	Content of lipids in blood serum, mg%				Amount of cholesterol removed from the body, mg	Degree of athero-sclerotic damage to the aorta, %
		Initial		At end of experiment			
		Cholesterol	Triglycerides	Cholesterol	Triglycerides		
1		19	80	426	76	876	40.7
2	Lipoextraction	39	90	534	264	1162	42.7
3		29	58	320	138	655	45.4
	M±m	29±8	76±13	427±90	159±79	898±213	42.7±2.0
4		25	75	392	75	–	44.5
5	Control	61	114	452	128	–	58.6
6		25	49	217	204	–	70.8
7		40	124	576	480	–	46.4
	M±m	38±10	90±11	409±101	222±11		55.1±7.4

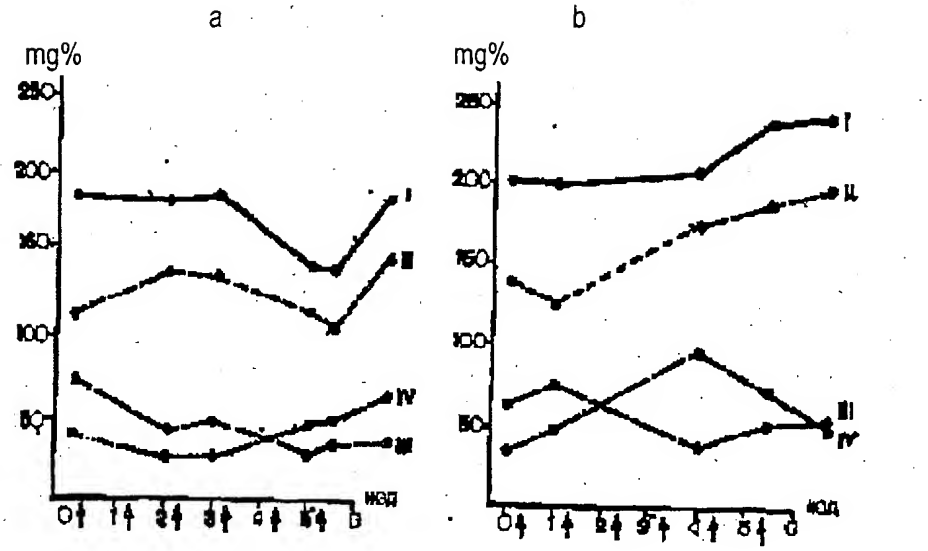
Repeated delipidization of blood plasma only led to a slight (statistically insignificant) reduction in the degree of atherosclerotic damage to the aorta in comparison with the control

animals, despite the fact that an average of about 900 mg cholesterol was removed from the body in each experimental rabbit as a result of lipid extraction.

In another series of experiments, we attempted to determine the effect of repeated lipoextraction of plasma on the regression of experimental atherosclerosis in rabbits (Table 2). Realizing that regression of atherosclerosis in rabbits continues for 1 year and more, we nevertheless calculated that the lipoextraction of plasma will accelerate regression; the effect can even be observed by the end of the second month of the experiment. As a result of lipoextraction from the blood plasma of each rabbit, we eliminated about 1300 mg of cholesterol. However, in these animals after lipoextraction we did not detect a significant change in the cholesterol concentration in the blood serum in comparison with the control: in the experimental rabbits the average cholesterol concentration was 262 mg% and in the controls it was 278 mg%. The degree of damage to the aorta in the experimental animals was 60%, and in the control was 70%. Thus, these studies conducted on animals who received large doses of cholesterol, although indicating the possibility of eliminating a certain part of it from the blood plasma by lipoextraction, on the whole did not enable us to find a significant effect of lipoextration on the course of experimental atherosclerosis and the initial stage of its regression in rabbits.

Table 2. Effect of lipoextraction of blood plasma on cholesterol content and triglyceride content in blood serum and degree of atherosclerotic damage to the aorta in rabbits during regression of the atherosclerotic process.

Number of rabbits	Experimental conditions	Content of lipids in blood serum, mg%			Amount of cholesterol removed from the body, mg	Degree of athero-sclerotic damage to the aorta, %
		Initial	At end of experiment			
		Cholesterol	Cholesterol	Triglycerides		
1	Lipoextraction	1420	356	64	1331	32.3
2		1470	168	80	1262	87.2
3	Control	1420	245	85	–	47.0
4		1440	300	44	–	93.1



Effect of lipoextraction of plasma on content of total cholesterol (I), high-density lipoprotein (II), and the total low-density and very low-density lipoprotein fraction (III) and triglycerides (IV) in blood plasma of dogs. Arrows – period of administration of delipidized plasma.

Apparently, the experimental model of atherosclerosis in rabbits is not suitable for answering the posed questions since this model, as already mentioned, is associated with administration to the animals of extremely high doses of cholesterol and oversaturation of the body with sterols. For delipidization of the plasma, each time we were only able to take a relatively small volume of blood (20 mL) from the ear vein of the rabbits; for this reason, the total amount of extracted lipids was not as high as we would have liked.

In conjunction with this, we set up experiments on two dogs without the preliminary administration of cholesterol (the level of lipids in the blood of dogs is normally much higher than in rabbits). In the experiments in dogs, we established one additional objective – to determine whether repeated intravenous administration of delipidized plasma leads to some favorable changes in the bodies of the animals. After six lipoextraction procedures, in the first dog, we withdrew 594 mg of cholesterol from the body and 501 mg in the second, which amounts to roughly ⅔ of the total amount of cholesterol in the blood plasma of dogs of this weight. The effect of repeated lipoextraction on the dynamics of lipid content in the blood plasma is shown in the figure. As a result of six lipoextractions, the concentration of total cholesterol in the blood plasma in the first dog diminished by 24%; in the second, no reduction was noted. However, in both dogs after delipidization of the plasma, we observed a reduction in cholesterol content of the total low- and very low-density lipoprotein fraction and an increase in

the content of high-density lipoproteins. Table 3 shows the dynamics of the content in blood plasma of a number of biochemical indices that reflect the state of metabolism in dogs, whose plasma was subjected to repeated delipidization. It is apparent from Table 3 that lipoextraction did not significantly change these indices. The increased content of urea in the blood plasma 1 week after catheterization of the jugular vein was apparently a reaction to the surgical intervention. The animals tolerated the procedure of intravenous administration of delipidized plasma well and they did not lose weight. No changes in behavior of the dogs were noted.

Table 3. Effect of lipoextraction of plasma on content of bilirubin, uric acid, urea, glucose, and aspartate-aminotransferase activity in blood plasma of dogs.

	Plasma sampling time	Bilirubin, mg%	Uric acid, mg%	Urea, mg%	Glucose, mg%	Aspartate aminotransferase activity, IU
Male dog 1	Before beginning of the experiment	0.08	0.70	64.0	125	12
	After delipidization:					
	2nd	0.08	0.75	39.2	117	14
	3rd	0.11	0.90	15.0	102	14
	5th	0.07	0.50	22.0	71	10
	1 day after the 6th delipidization	0.07	0.90	34.0	116	22
	10 days after the 6th delipidization	0.08	0.60	26.2	135	24
Female dog 2	Before beginning of the experiment	0.20	0.80	55.2	122	10
	After delipidization:					
	1st	0.08	1.10	9.6	88	18
	4th	0.06	0.40	10.1	100	11
	1 day after the 6th delipidization	0.09	0.80	7.0	114	26
	10 days after the 6th delipidization	0.10	0.80	10.8	105	15

Thus, the performed study demonstrated the possibility of eliminating cholesterol from blood plasma by repeated delipidization. The absence of a fairly pronounced effect of repeated lipoextractions on the level of lipids in the blood and degree of damage to the aorta in rabbits, in all likelihood, stems from the administration of very high doses of cholesterol to these animals. In the experiments in dogs, we observed a reduction in the content of cholesterol in the blood of the atherogenic low- and very low-density lipoproteins and an increase in the content of cholesterol of the high-density lipoproteins. These changes in cholesterol content reflect a change in concentration of the actual lipoproteins in the blood. High-density lipoproteins, as already mentioned, have anti-atherogenic properties, for which an increase in the content of lipoproteins of this class in dogs as a result of lipoextraction of the blood plasma should be viewed as a favorable change in the lipoprotein spectrum. The experiments also showed that this procedure is harmless and can be used to reduce the lipid level in the blood and possibly to eliminate part of the cholesterol from the arterial wall.

CONCLUSIONS

1. The method of delipidization of plasma with diethyl ether permits the elimination of mostly triglycerides, cholesterol, and only one-third of the phospholipids.
2. In experiments on rabbits with experimental hypercholesterolemia, by repeated lipoextraction of small volumes of plasma (12-14 mL each), it was possible to remove 900-1300 mg of cholesterol from the body.
3. In normal dogs, as a result of six lipoextraction procedures of the plasma (70-90 mL each), the content of cholesterol of the total atherogenic lipoprotein fraction diminished in the plasma and the content of cholesterol of the anti-atherogenic high-density lipoproteins increased.
4. The lipoextraction procedures are harmless and can be used to reduce the level of lipids in blood and possibly eliminate some cholesterol from the arterial wall.

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